FINAL TECHNICAL REPORT

Grant#: N00014-92-J-1710

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INSTITUTION: University of Illinois

GRANT TITLE: Biofilm Structure and Diversity

OBJECTIVE: To develop molecular measures of the identity, activity, and spatial distribution of microbial populations making up natural (multispecies) biofilms. To use biofilm bioreactors to characterize the relationship between biochemical transformations and community structure, focusing on the terminal steps of anaerobic mineralization and emphasizing competitive and cooperative interactions among sulfate-reducing and methanogenic biofilm populations. Long term objectives include the identification and characterization of obligate symbioses among attached microbial populations and the refinement of mathematical models to predict the behavior of biofilm communities.

ACCOMPLISHMENTS

Use of Fluorescent Probes for Assessing Single Cell Activity: During this project period we completed a study on the use of fluorescent probes to measure the activity of individual cells of a desulfovibrio population in developing multispecies biofilms. Two papers, one describing this work and a second demonstrating the use of oligonucleotide hybridization probes to monitor the enrichment and isolation of a biofilm population initially identified by rRNA sequence, were published (Kane et al. 1993, Poulsen et al. 1993).

New Biofilm Reactor Design. Current reactor design is not well suited to reproducible sampling of biofilm specimens for microscopic examination. This is because biofilm of varying age and thickness develops on the glass bead substratum and in the interstitial spaces separating the glass beads. In order to better control development and sampling of biofilm we are currently evaluating a new bioreactor design (Figure 1). This is a fluidized bed reactor that uses small sections of polyethylene tubing as substratum. The fluidized substratum provides a relatively uniform and protected surface (the inside of the tubing) for microbial colonization and biofilm development. The tubing can be serially sectioned (2 - 10 um slices) to facilitate microscopic examination, for example following hybridization with fluorescent probes.

Use of multiple fluorescent probes to determine the spatial distribution of methanogenic and SRB populations. We are continuing to evaluate the the use of simultaneous hybridization with group-specific fluorescent probes labeled with different fluorescent dyes (targeting methanogens, sulfate-reducing bacteria (SRB), and the archaeal and bacterial domains) to define spatial relationships among different physiological groups. The goal of these studies is to identify close physical associations that could reflect close physiological associations

among major biofilm populations. Methanogen DNA probes, including those probes specific for the morphologically distinct Methanosaeta and Methanosarcina species, were demonstrated for use in whole cell hybridization to multispecies biofilm samples.

Biofilm response to altered sulfate availability and acetate addition. Extensive studies of the last 12 months demonstrated that the SRB (primarily Desulfovibrio species) comprise a large fraction of the biofilm community; BOTH in the presence and absence of sulfate (approximately 35% and 25% respectively). This suggests that SRB play a much greater role in low sulfate systems than previously thought. At low sulfate, methanogens presumably serve as an electron sink for the SRB (via hydrogen consumption). However, this begs the question of electron donor(s) used by the desulfovibrio in the absence of sulfate (since they are a major hydrogen consuming population in its presence. Preliminary results of acetate addition experiments show an increase in desulfovibrio numbers and suggest that acetate could be a substrate for desulfovibrio under low sulfate conditions.

Mathematical modeling. Two preliminary models for the reactor systems were previously developed, one in the absence of sulfate and the other in the presence of non-limiting sulfate concentrations. The models are currently being used to predict biofilm population response to exogenous acetate.

Distribution of sulfidogens in a sulfate-dominated mat community. In a collaborative study with Dr. James Risatti (University of Illinois, Natural History Survey), group- and species-specific hybridization probes developed for ONR sponsored research were used to quantify specific populations of sulfate-reducing bacteria in a hypersaline microbial mat from Baja California, Mexico. This study revealed that SRB populations in this community are highly stratified. Populations of Desulfococcus/Desulfosarcina/ Desulfobotulus and Desulfovibrio were located near the surface of the mat (1-13 mm) whereas Desulfobacterium and Desulfobacter species were restricted to partly overlapping intervals at depths of approximately 34-42 mm. These observations suggest that the phylogenetically defined probe target-groups correspond to assemblages that serve well defined ecological/functional roles in anaerobic mineralization.

SIGNIFICANCE

Control and manipulation of biofilms should beneflit from understanding of community architecture. The observation of stratification of sulfidogens in sulfate-dominated microbial mats supports the use of probes targeting phylogenetically-defined microbial groups to resolve populations that serve different roles in anaerobic mineralization. The observation that sulfate-reducing bacteria persist at high numbers within sulfate-depleted biofilms could significantly change existing models of anaerobic mineralization and greatly expand the role of SRB in these processes. This could have important implications for predicting the distribution and activity of SRB in natural and managed biofilm communities. For example, the observation that sulfate-reducing bacteria are abundant both in the presence and absence of sulfate should be considered in attempts to control their numbers or activities in biofilms.

PUBLICATIONS AND REPORTS

1. Journal Articles (Publications related to this granting period).

Kane, M.D., Poulsen, L.K. and Stahl, D.A. 1993. Monitoring the enrichment and isolation of sulfate-reducing bacteria by using oligonucleotide probes designed from environmentally-derived 16S rRNA sequences. Appl. Envrion. Microbiol. 59: 682-686.

Poulsen, L.K., G. Ballard, and D.A. Stahl. 1993. Use of Fluorescence in situ hybridization for measuring the activity of single cells in young and established biofilms. Appl. Environ. Microbiol. 59: 1354-1360.

2. Manuscripts prepared for submission

Raskin, L., Stromley, J.M., Rittmann, B.E. and Stahl, D.A. 16S rRNA hybridization probes to describe natural communities of methanogens.

3. Abstracts of Meeting Presentations

Poulsen, L., Capman, W., V. Kirvelaitis, J. Risatti and D.A. Stahl. 1993. The use of nucleic acid probes to study the activity and structure of biofilm and microbial mat populations. Abstracts of ASM Conference on Multicellular and Interactive Behavior in Bacteria. Woods Hole, Massachusetts. March 28-April 1, 1993. Woods Hole Massachusetts.

Poulsen, L. and Stahl, D.A. 1993. Use of video microscopy to measure the activity of single cells in early and established biofilms. Abstracts of ASM Conference on Multicellular and Interactive Behavior in Bacteria. Woods Hole, Massachusetts. March 28-April 1, 1993. Woods Hole Massachusetts.

4. Invited Participant

American Academy of Microbiology Colloquium on Strategies and Mechanisms for Field Research in Environmental Bioremediation. San Antonio, Texas, January 8-10, 1993.

ASM Conference on Multicellular and Interactive Behavior in Bacteria. Woods Hole Massachusetts, March 28 - April 1, 1993.

Marine Sediment Biogeochemistry Workshop. Sponsored by the Office of Naval Research in association with the Federation of American Societies for Experimental Biology. Washington, D.C. May 26-28, 1993.

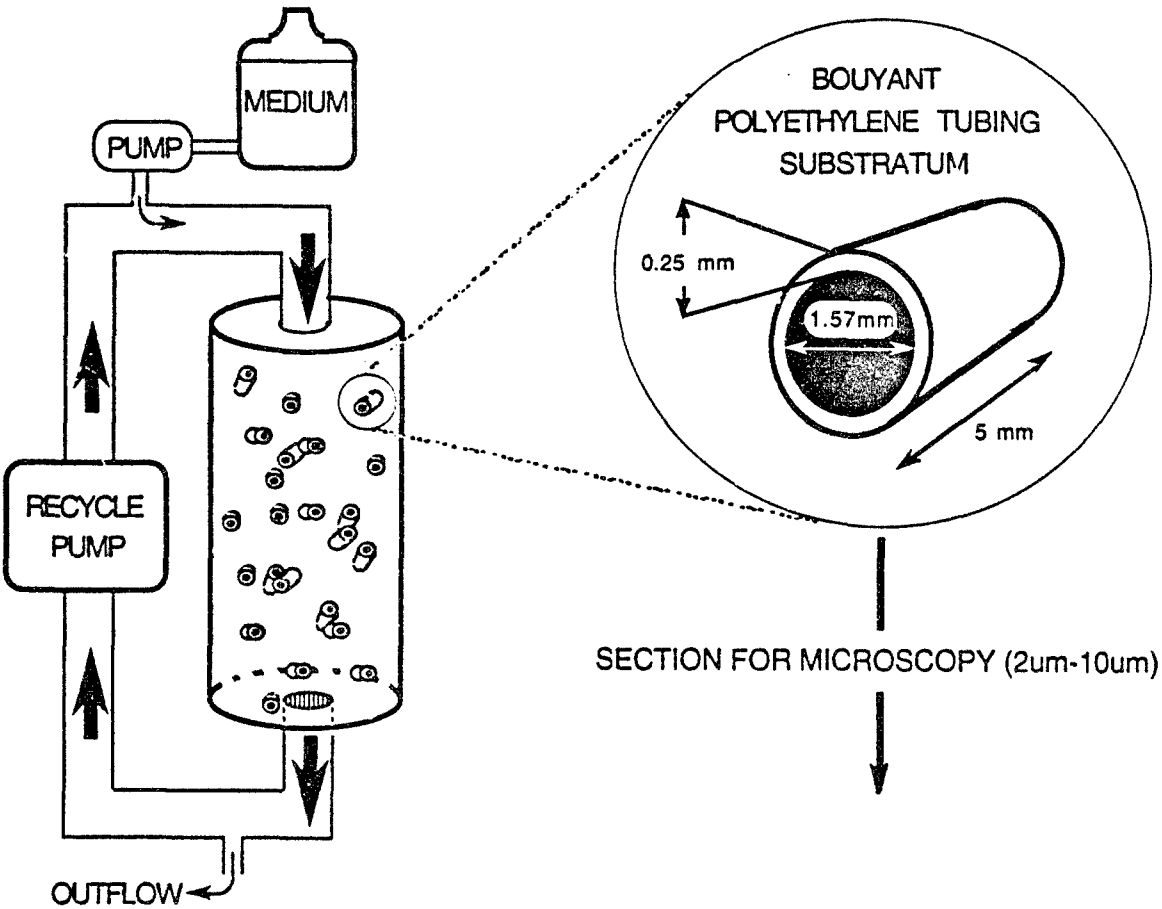
FIGURES

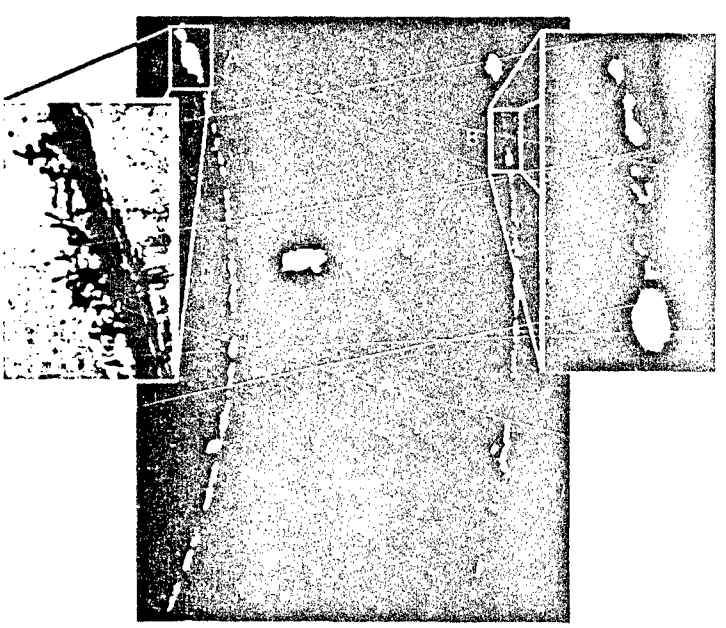
Figure 1

Fluidized bed bioreactor and microscopic examination of attached biofilm. The reactor is designed for reproducible sampling and microscopic examination of biofilm. The inside surface of the bouyant polyethylene tubing provides a protected substratum for microbial colonization. The tubing can be sectioned (2 to 10 microns thick) and hybridized with fluorescent probes prior to microscopic examination.

Lower right. Image of a DAPI-stained biofilm colonizing the tubing surface from a methanogenic reactor (2 um parafin section). Phase contrast (left) and fluorescent (right) images are shown in enlargement).

FLUIDIZED BED BIOFILM REACTOR





API STAINED SECTION
A) Phase Contrast
B) Fluorescence